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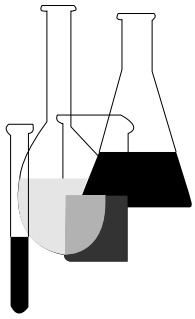
Health Effects Test Guidelines

OPPTS 870.5575
Mitotic Gene Conversion in *Saccharomyces cerevisiae*



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5575 Mitotic gene conversion in Saccharomyces cerevisiae.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5575 Mitotic gene gene conversion in Saccharomyces cerevisiae and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** The mitotic gene conversion assay in the yeast, *Saccharomyces cerevisiae* (*S. cerevisiae*), measures the conversion of differentially inactive alleles to wild-type alleles by mutagenic agents. Heteroallelic diploid yeast strains carry two different inactive alleles of the same gene locus. The presence of these alleles causes a nutritional requirement, e.g., these heteroallelic diploids grow only in medium supplemented with a specific nutrient such as tryptophan. When gene conversion occurs, a fully active wild-type phenotype is produced from these inactive alleles through intragenic recombination. These wild-type colonies grow on a medium lacking the specific nutritional requirement (selective medium).

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Heteroallelic diploids are diploid strains of yeast carrying two different, inactive alleles of the same gene locus causing a nutritional requirement.

Mitotic gene conversion is detected by the change of inactive alleles of the same gene to wild-type alleles through intragenic recombination in mitotic cells.

(d) **Reference substances.** These may include, but need not be limited to, hydrazine sulfate or 2-acetylaminofluorene.

(e) **Test method**—(1) **Principle.** The method is based on the fact that heteroallelic diploid yeast strains carry two inactive alleles of the same gene locus making them dependent on a specific nutritional requirement (e.g., tryptophan) for their survival. Treatment of such strains with mutagenic agents can cause conversion of these alleles back to the wild-type condition which allows growth on a medium lacking the required nutrient (selective medium).

(2) **Description.** Heteroallelic diploid strains such as D7, requiring a specific nutrient in the medium, are treated with test chemical with and without metabolic activation and plated on a selective medium lacking the required nutrient. The wild-type colonies that grow on the selective medium as a result of gene conversion are scored.

(3) **Strain selection**—(i) **Designation.** At the present time, *S. cerevisiae* strain D7 is recommended for use in this assay. The use of other strains may also be appropriate.

(ii) **Preparation and storage.** Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) **Media.** YEP glucose medium enriched with the appropriate growth factors may be used for cell growth and maintenance. Other media may also be appropriate.

(4) **Selection of cultures.** Cells should be grown with aeration in liquid medium enriched with growth factors to early stationary phase. Cells should then be seeded on selective medium to determine the rate of spontaneous conversion. Cultures with a high rate of spontaneous conversion should be discarded.

(5) **Metabolic activation.** Cells should be exposed to test chemical both in the presence and absence of an appropriate metabolic activation system.

(6) **Control groups.** Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) **Test chemicals**—(i) **Vehicle.** Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay. Dimethylsulfoxide should be avoided as a vehicle.

(ii) **Exposure concentrations.** (A) The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. For cytotoxic chemicals, the highest dose tested should not reduce survival to less than 10 percent of that seen in the untreated control cultures. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case-by-case basis.

(B) When appropriate, a positive response should be confirmed by using a narrow range of concentrations.

(f) **Test performance**—(1) **Treatment.** Cultures should be treated in liquid suspension. Resting cells should be treated in buffer; growing cells should be treated in a synthetic medium. Cultures with low spontaneous convertant frequencies should be centrifuged, washed and resuspended in liquid at the appropriate density. Cells should be exposed to test chemical both in the presence and absence of a metabolic activation system. Independent tubes should be treated for each concentration. At the end of the treatment period, cells should be centrifuged, washed and resuspended in distilled water prior to plating on selective medium for convertant selection and on complete medium to determine survival. At the end of the incubation period, plates should be scored for survival and the presence of convertant colonies.

(2) **Number of cultures.** At least six individual plates per treatment concentration and control should be used.

(3) **Incubation conditions.** All plates in a given experiment should be incubated for the same time period. This incubation period may be from 4 to 6 days at 28 °C.

(g) **Data and report**—(1) **Treatment of results.** Individual plate counts for test substance and control should be presented for both convertants and survivors. The mean number of colonies per plate and standard deviation should also be presented. Data should be presented in tabular form indicating numbers of viable and convertant colonies scored, survival frequency and convertant frequencies for each treatment and control culture. Conversion frequencies should be expressed as number of convertants per number of survivors. Sufficient detail should be provided for verification of survival and convertant frequencies.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of gene convertants. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of gene conversions or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results in this assay indicate that under the test conditions the test chemical causes mitotic gene conversion in the yeast *S. cerevisiae*.

(ii) Negative results indicate that under the test conditions the test chemical does not cause mitotic gene conversion in *S. cerevisiae*.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used, and rationale for dosage selection.

(iii) Method used to select cultures.

(iv) Treatment protocol including cell density at treatment and length of exposure to test substance.

(v) Details of both the protocol used to prepare the metabolic activation system and its use in the assay.

(vi) Incubation times and temperatures.

(vii) Dose-response relationship, if applicable.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Ames, B.N. et al. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research* 31:347–364 (1975).

(2) Callen, D.F. and Philpot, R.M. Cytochrome P–450 and the activation of promutagens in *Saccharomyces cerevisiae*. *Mutation Research* 45:309–324 (1975).

(3) Zimmermann, F.K. Procedures used in the induction of mitotic recombination and mutation in the yeast *Saccharomyces cerevisiae*. Handbook of mutagenicity test procedures. Eds. Kilby, B.J., Legator, M., Nicols, W., Ramel, C. Elsevier/North Holland Biomedical Press, Amsterdam (1979) pp. 119–134.

(4) Zimmermann, F.K. et al. A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion and reverse mutation. *Mutation Research* 28:381–388 (1975).